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A STARCH HYDROLYSIS PROCEDURE TO ESTIMATE GLYCEMIC INDEX

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ABSTRACT

An *in vitro* procedure to measure the rate of starch digestion in starchy common foodstuffs was developed. A first-order equation that rules the hydrolytic process was found: $C=C_\infty\ (1-e^{-kt})$. Besides an *in vivo* assay, to calculate the glycemic index (GI), was carried out on thirty healthy volunteers. This is a simple *in vitro* method that could be used to estimate the metabolic glycemic response to a food. The best correlated value with *in vivo* glycemic responses was the percentage of starch hydrolysis at 90 min ($r= 0.909$, $p\leq 0.05$, $GI_1= 39.21+ 0.803(H_{90})$).

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Key words: Starch Hydrolysis, Glycemic Index, Starchy Foods.

INTRODUCTION

The rate of starch digestion and absorption seems to be a determinant of the metabolic response to a meal (1). There are evidences that slowly digested and absorbed carbohydrates are favourables in the dietary management of metabolic disorders, such as diabetes and hyperlipidemia (2,3) . Foods such as legumes, pasta and whole grain cereals are supposed to have this effect. They are called "lente" carbohydrates. Many factors can influence the kinetics of starch digestion, such as the nature of starch, physical form, protein and lipids interactions, presence of antinutrients and enzyme inhibitors, food processing (4).

Jenkins et al (5) introduced the concept of glycemic index (GI) to classify foods on the basis of their postprandial blood glucose response. The GI is defined as the postprandial incremental glycemic area after a test meal, expressed as the percentage of the corresponding area after an equi-carbohydrate portion of a reference food (glucose or white bread). Studies of postprandial glucose demand several subjets during a long period of time, are laborious and the facilities necessary for this kind of study are not always present in food research laboratories. The interest in an *in vitro* methodology to estimate this glycemic response has recently increased.

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Several *in vitro* procedures (6–13) have been proposed to evaluate the rate of starch hydrolysis considered as a predictor of the physiological effects of a particular meal. There is no agreement about the procedure or the results. Sample preparation is different in each particular method. Some of these methods used only amylases, whereas others used proteolytic enzymes in combination with amylases. Time points to establish a good relationship with *in vivo* data are also different from one method to another. The hydrolysis may be performed unrestricted or restricted (dialysis). On the other hand, some have failed to predict *in vivo* properties, in the case of legumes.

Taking into account the lack of a common *in vitro* starch hydrolysis procedure to estimate the GI, the aim of this work was to find an *in vitro* method, well correlated with the *in vivo* responses to the same food.

METHODS AND MATERIALS

PRODUCTS

Common dietary starchy foods were chosen. Cereals: white bread, spaghetti, rice and biscuits. Legumes: lentils, chick-peas and beans. Vegetables: peas, boiled potatoes and crisp potatoes. Products were purchased in local markets.

Corn starch (Sigma S-4126) was used as a standard to establish the *in vitro* procedure to measure the kinetics of starch digestion.

All samples (50mg), except for white bread, biscuits and crisp potatoes, were boiled in tap water (5mL) in capped tubes until edible (cooking time, according to the mediterranean tradition, ranging from 15 min for pasta to 90 min for beans). Samples with more than 5% of fat (biscuits and crisp potatoes) were defatted using Soxhlet extraction with petroleum ether (Soxhlet System HT Tecator). Sample preparation and analysis was carried out in the same tube. They were homogenized in liquid medium, either water in boiled samples or buffer in the raw ones using a homogenizator (POLYTRON®, PCU KINEMATICA GmBH, Switherland) with controlled speed (level 2 in the power control unit for 1 min).

STARCH ANALYSIS

Total Starch (TS).

Quadruplicated samples of 50 mg were dispersed in 6mL of 2M KOH and energically shaken at room temperature for 30 min. 3 ml of 0.4M Sodium acetate buffer pH=4.75 and 60 μl of amyloglucosidase (Ref 102 857, Boehringer) were added to this suspension and incubated for 45 min at 60°C in a controlled shaking water bath. Starch was measured as glucose with Peridochrom Glucose GOD-PAP (Ref 676 543, Boehringer). Factor conversion from glucose to starch was 0.9.

Resistant Starch (RS) and Digestible Starch (DS).

RS content in the food was measured by Goñi et al method (14). In brief, the main steps of the procedure are: protein removal from samples with pepsin (Art.7190, Merck);

α-amylase (E.C.3.2.1.1. Type VIB from porcine pancreas, Sigma) incubation for 16 hours to hydrolyze digestible starch; treatment of the residue with 2M KOH to solubilize resistant starch; incubation with amyloglucosidase and determination of glucose using a glucose oxidase assay (GOD-PAP reagent). RS was calculated as glucose (mg) x 0.9. DS has been calculated by difference between TS and RS.

IN VITRO KINETIC OF STARCH DIGESTION.

An improved *in vitro* method, standarized in our laboratory, was used to measure the rate of starch hydrolysis at different times.
Procedure: Food portions of 50 mg were prepared as explained above. 10 mL of HCl-KCl buffer pH=1.5 were added (pH was adjusted). Then 0.2 mL of a solution containing 1 g of Pepsin in 10 mL of HCl-KCl buffer were added to each sample and incubated at 40°C for 1 hour in a shaking water bath. Volume was completed to 25 mL with Tris-Maleate buffer pH=6.9. 5 mL of a solution of α-amylase in Tris-Maleate buffer containing 2.6 UI were added to each sample. Samples were then incubated at 37°C in a shaking water bath. 1mL aliquot samples were taken from each tube every 30 min from 0 to 3 hours. These aliquots were placed in a tube at 100°C and were energically shaken for 5 min to inactivate the enzyme and refrigerated until the end of the incubation time. Then 3 mL of 0.4M Sodium acetate buffer pH=4.75 were added to each aliquot, and 60μL of amyloglucosidase were used to hydrolyse the digested starch into glucose after 45 min at 60°C in a shaking water bath. Volume was adjusted to 10-100 mL with distilled water. Triplicated aliquots of 0.5 mL were incubated with Peridochrom Glucose GOD-PAP (Ref 676 543, Boehringer). The glucose was converted into starch by multiplying for 0.9.

The rate of starch digestion was expressed as the percentage of TS hydrolysed at different times (30, 60, 90, 120 and 180 min).

The areas under hydrolysis curves (AUC, 0-180 min) were calculated, with the equation described below, for all products. The HI was calculated as the relation between the AUC for a food and the AUC for a reference food, white bread, expressed as a percentage (11).

To study the possible correlation between *in vitro* and *in vivo* responses to the same food, the HI obtained with this *in vitro* procedure and the GI_1 reported in The International Tables of GI(15) were compared. The percentage of starch hydrolysis at 30, 60, 90, 120 and 180 min and GI_1 were also compared.

IN VIVO STUDY.

There are regionals differences in foods, so we have measured the glycemic response to typical mediterranean foods to compare the results with the GI from the International Tables.

Subjects: Thirty healthy volunteers participated in the study (22 women and 8 men) with mean age 23.8±3.9. Their mean body mass indices were normal 21.0±1.9 Kg/m^2. Volunteers gave their informed consent. The study was approved by the Department of Nutrition of the Complutense University, Madrid. All subjects consumed white bread, considered as the standard food. They were divided into five groups of six persons and each group consumed one of the chosen foods. The study was conducted after a 12 hours fasting. The foods were given between 9:30 and 10:00 and were eaten over 15 min.

Fingerprick blood samples were taken using Softclix-pro (Boehringer) in the fasting state and 15, 30, 60, 90, 120 and 180 min after the meal. Blood samples were taken on test scripts and analysed with Accutrend® glucose (Boehringer). The GI_2 was calculated following the procedure of Wolever et al (16). These values were compared with the HI and the GI_1 from the literature.

Foods: The volunteers took foods containing 50 g of starch, according to data in Table 1, from white bread, spaghetti, rice, biscuits and crisp potatoes. In the case of lentils they were given the equivalent to 25 g of starch and white bread was also adapted to this amount of starch in these subjects, as recommended when the amount of food to be intaken was too much (17). Spaghetti, rice and lentils were boiled in salted water. Volunteers were allow to drink water, 150–300 mL depending on the food.

STATISTICAL ANALYSIS.

The results are reported as mean ± SD. The equation for the display of the hydrolysis curves was calculated by using the computer systems SAS/STAT Version 6.0 SAS Institute, USA and Mathematica 2.2. Linear regression analysis have been performed to compare HI with GI_1, HI with GI_2, GI_2 with GI_1 and finally the percentage of starch hydrolysis at different sampling times with GI_1 from Brand Miller,1995 and GI_2, experimental values by using a Statgrafics computer system.

RESULTS

Experimental values for TS and starch fractions (RS and DS) are reported in Table 1.

TABLE 1

Total Starch (TS), Resistant Starch (RS) and Digestible Starch (DS) in Starchy Foodstuffs (mean±SD, % Dry Matter).

Food (Moisture %)	TS	RS	DS*
Bread (37.7)	76.70±2.16	2.49±0.57	74.2
Spaghetti[1] (7.4)	74.00±2.24	2.92±0.64	71.08
Rice[1] (12.9)	82.22±2.24	2.53±0.68	79.69
Biscuits[2] (4.2)	62.89±2.09	1.59±0.18	61.3
Lentils[1] (10.0)	41.69±4.15	6.83±0.06	34.83
Chickpeas[1] (8.4)	45.08±3.67	4.36±0.35	40.62
Beans[1] (13.4)	31.66±3.72	5.48±0.62	26.18
Frozen peas[1] (85.8)	18.93±1.89	10.03±0.40	8.9
Boiled potatoes[1] (76.9)	57.76±8.95	1.00±0.14	56.76
Crisp potatoes[2] (2.6)	65.42±2.50	3.27±0.79	62.15

* DS has been calculated as the difference between TS and RS.
[1] Samples boiled in tap water before being analysed.
[2] Deffated.

Hydrolysis curves for each product are diplayed in FIG 1. The curves follow a first order equation:

$$C=C_{\infty}\ (1-e^{-kt})$$

where C is the concentration at t time, C_{∞} is the equilibrium concentration, k is the kinetic constant and t is the chosen time.

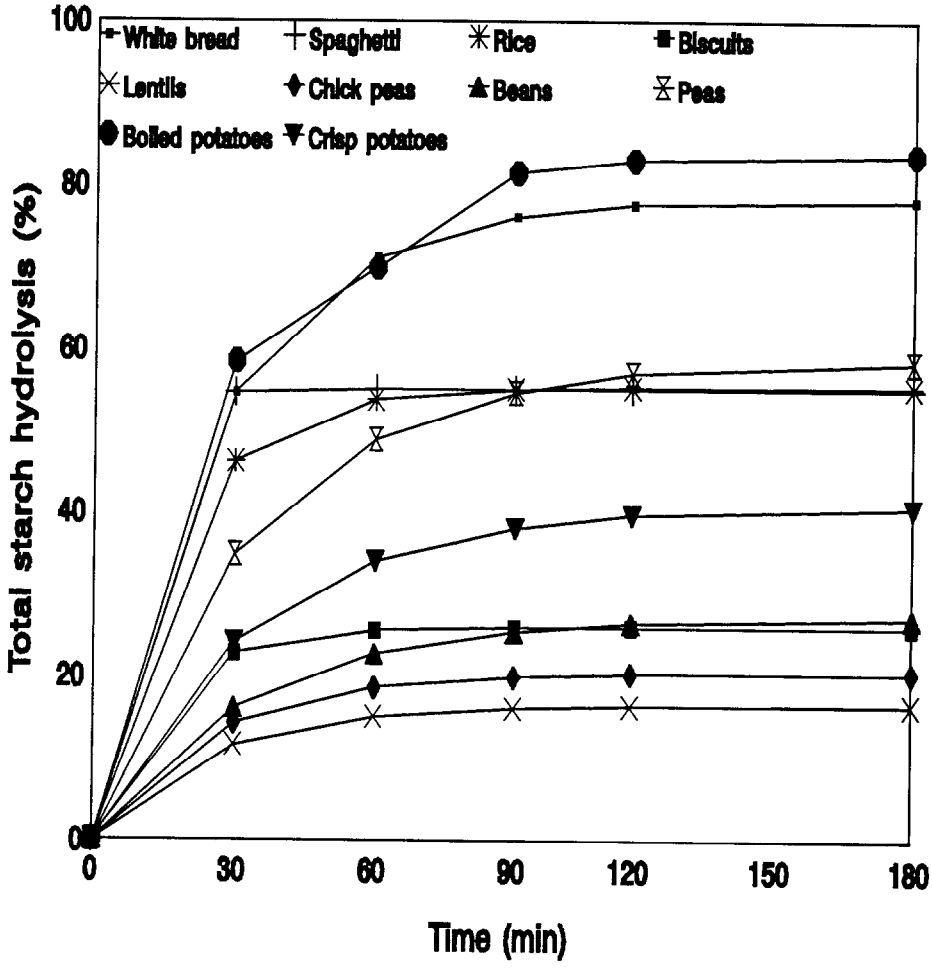

FIG 1. Total Starch Hydrolysis Rate.

This equation was proved with corn starch as standard. Different concentrations were analysed to assure the fit to this exponential model. Experimental values for each food were adjusted to the function to calculate k and C_∞ (Table 2), and the whole equation was used to draw the graphics.

Differences between groups of products can be apreciated. Legumes generate less percentage of hydrolysis, whereas the hydrolysis value for cereals is always higher. The curves show a first part from 0 to 90 min where the hydrolysis rate increases and a second from 90 to 180 min where a maximal plateau level is slowly reached.

White bread was used as the reference food. Previous works (11) have found nearly 50% of digestion within 180 min. while our result was 76.1%. This could be explained due to the different methodologies used. We have included pepsin in the hydrolysis, and used an unrestricted system.

The AUC was calculated as the integral of the kinetic equation, and used to obtain the HI for each food, which values are reported in Table 2.

TABLE 2

Percentage of Total Starch hydrolysed at 90 min (H_{90}), Equilibrium Concentration (C_∞), Kinetic Constant (k) and Hydrolysis Index (HI) for Starchy Foods.

Food	H_{90exp} **	H_{90thc} **	C_∞ *	k*	HI
White bread	74.9±7	76.1	78.19	0.04	100
Spaghetti	51.3±1	55.1	55.09	0.16	82±2
Rice	55.1±3	55.1	55.34	0.06	77±1
Biscuits	25.4±1	25.9	25.94	0.07	37±1
Lentils	14.8±3	16.1	16.59	0.04	22±6
Chickpeas	18.4±2	19.9	20.46	0.04	27±5
Beans	24.7±9	25.4	27.20	0.03	34±8
Peas	52.1±12	54.7	58.61	0.03	76±7
Boiled potatoes	75.2±15	81.4	83.71	0.04	112±18
Crisp potatoes	36.9±11	37.9	40.65	0.03	51±12

* parameters of the kinetic equation $C=C_\infty (1-e^{-kt})$
** H_{90exp} experimental value, H_{90thc} theoric value obtained with the equation.

The glycemic indeces (GI_2) obtained in the *in vivo* assay are listed in Table 3. The values were similar to those referenced in the GI International Tables (15). A good correlation was found between GI_1 and GI_2 $r= 0.952$, $p\leq 0.05$.

TABLE 3

Glycemic Index (GI_1 ,referenced, GI_2 experimental) of Starchy Foodstuffs.

Food	GI_1	GI_2
White bread	100	100
Spaghetti	78±7	68±17
Rice	81±3	88±16
Biscuits	79±9	87±12
Lentils	42±6	38±7
Chickpeas	47±9	nd
Beans	60±6	nd
Peas	74±3	nd
Boiled potatoes	101±2	nd
Crisp potatoes	74±4	81±7

nd: not determined.

Mean blood glucose responses in mg/dL during 120 min following the ingestion of test foods are shown in FIG 2. The highest peaks were found for rice and spaghetti. Lentils as it was expected gave the lowest glucose response.

The possible estimation of GIs from the percentage of total starch hydrolysed, depending on sampling time, was also analysed. Table 4 shows the correlation coefficients obtained by linear regression $p \leq 0.05$. According to these results the best hydrolysis value to estimate *in vivo* glycemic response would be 90 min (H_{90}), $r = 0.909$, $p \leq 0.05$, $GI_1 = 39.21 + 0.803(H_{90})$.

Good, but lower, linear correlation was found between the HI and the GI_1, $r = 0.894$, $p \leq 0.05$ following the equation $GI_1 = 39.71 + 0.549HI$.

TABLE 4

Correlation Coefficient (r) between the Percentage of Hydrolysis at different time and the Glycemic Index (GI_1) from the literature (15).

Time	r	p
30 min	0.836856	0.00253
60 min	0.883504	0.00070
90 min	0.908806	0.00027
120 min	0.889978	0.00056
180 min	0.887689	0.00061

p= probability level

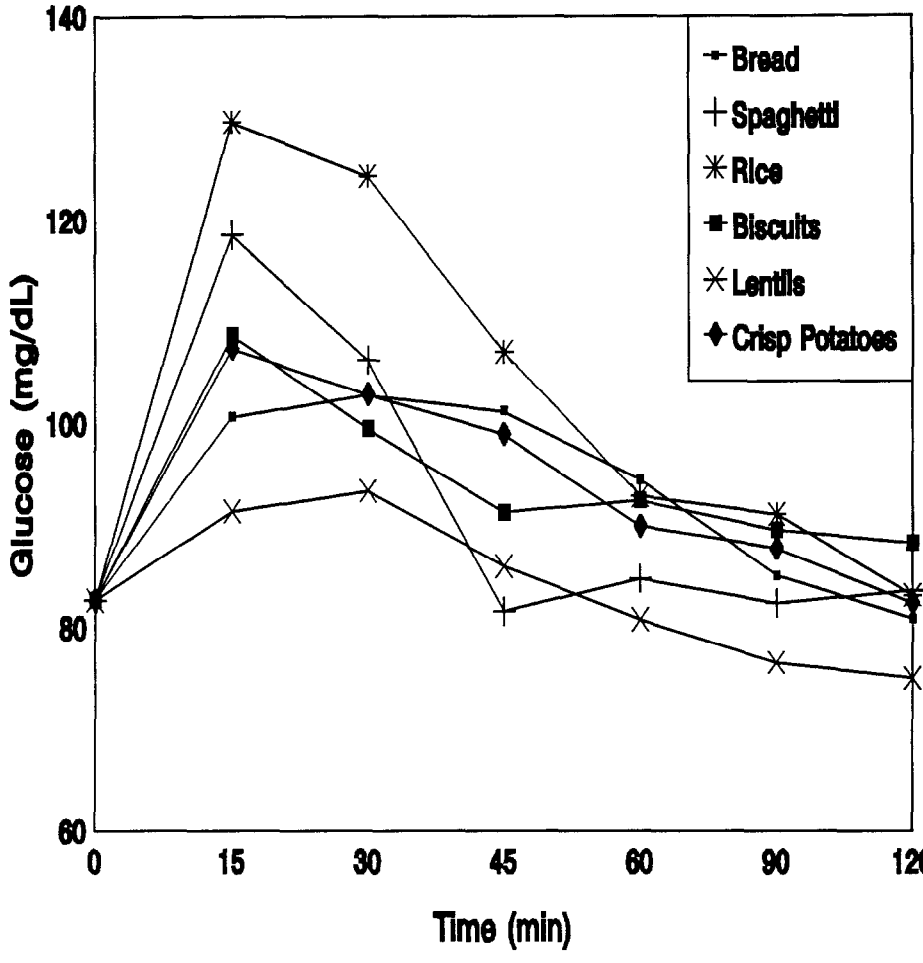

FIG 2. Blood Glucose Response after 50 g Starchy Foods.

DISCUSSION

A simple *in vitro* procedure has been described, in which the rate of hydrolysis of starchy foods could be properly measured. It could be useful to estimate the metabolic glycemic response to a food.

There are several differences between the present method and previous ones. Sample preparation (i.e. destruction of food structure) is different in each method. Milling, chewing, mincing and homogenizing are the most common procedures. Milling and mincing are used before cooking, but foodstuffs are usually destroyed after cooking, except in samples consumed without cooking. This fact may modify the rate of *in vitro* hydrolysis. In

the case of chewing, it could have many variations between and within individuals, it has been confirmed that different degrees of chewing can cause variation in the glycemic response (18). We propose the homogenization in liquid of the sample, raw or boiled depending on the product.

It is of importance that the *in vitro* procedure let us simulate starch enzymatic digestion at the best possible rate. In the procedure decribed in this work a protease (pepsin) to avoid protein-starch interactions (19) and α-amylase to hydrolyse starch were used, finally amyloglucosidase should release glucose from the starch hydrolysis products. The already proposed methodologies differ in the type of enzyme used, thus in some cases only amylase is used (6-8), in others it is combined with proteases (9,11).

Another difference among methods is that the enzyme incubation may be performed restricted i.e. employing dialysis (6,11) or unrestricted (7,8). We have chosen an unrestricted system in a capped tube which is more related to the digestion procedure than to the absorption one. The restricted procedure employing dialysis is in connection with absorption, because what is measured is the glucose in the dialysate, which has passed through a membrane.

With the present kinetic method it has been possible to adjust the hydrolytic process to the equation which draws the curve followed by each assayed food. The process has been adjusted to a first order model. It has been already reported that the Michaelis-Menten model was sufficient for describing kinetics at low starch concentrations and a modified first-order model was required at high concentrations (20). Previuos papers dealing with starch hydrolysis in relation to glycemic responses, do not give the equation which rules the kinetic of starch hydrolysis. The equation can be determined with only two experimental values and allows the prediction of the percentage of total starch hydrolysis at any time (i.e. there is a good agreement between theoric and experimental H_{90} values, Table 2). On the other hand from the integration of the equation the HI can be calculated. Each food has its own k and C_∞ , reported in Table 2.

The course of starch hydrolysis is characteristic for each product. There is a wide variation between food products in α-amylase susceptibility, from the lowest for lentils (18.95 % at 180min), to the highest for boiled potatoes (87.79% at 180min). Groups of food products have also differences, cereals showed higher digestion rate than legumes. There are several studies about why legumes are slowly digested, physical form , dietary fiber and protein content are some of the responsible factors proposed (21) but any satisfactory explanation has been already found. Legumes due to this characteristic have been shown to improve the lipoprotein profile (2).

Our *in vitro* results, displayed in Figure 1, agree with *in vivo* data showing low glycemic responses for legume products and high glycemic responses for cereal products (22).

On the basis that experimental GI_2 is similar to GI_1, we have compared *in vitro* data with GI_1. A good correlation (r=0.894) was found with the HI obtained from the experimental assay and the GI_1. The degree of hydrolysis within 30 min has been found to be well correlated with *in vivo* data (7,8,10). We could not find such good relationship at 30 min, indeed it was the value with the lowest correlation (Table 4). However, this correlation was better when the percentage of starch hydrolysis at 90 min (H_{90}) was compared with the GI_1 ($r= 0.909$, $p\leq 0.05$). The use of H_{90} would simplify calculation, avoiding the integration of the

curve and would also lessen experimental time, because this value is just one point of the hydrolytic process, taking one aliquot at 90 min and measuring the glucose content, the GI of the food could be estimated.

We conclude that the described *in vitro* procedure could be useful in the estimation of the GI. Furthermore, a mathematical first-order equation characteristic of the hydrolysis of each foodstuff was obtained.

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